Discussion

Several independent lines of evidence implicate PPARδ in psoriasis. PPARδ is highly, and specifically upregulated in psoriasis. The activity profile of this nuclear hormone receptor includes anti-apoptotic and pro-angiogeneic effects, as well as regulation of keratinocyte differentiation. PPARδ is induced by TNFα, a central mediator of the inflammatory response in psoriasis. Its interaction partners FABP5 and CRABPII are up-regulated in psoriasis, as well as a large group of dysregulated genes in psoriasis lesions *in vivo* related to PPARδ function (Romanowska *et al.*, 2007). In this thesis, it is shown that PPARδ enhances keratinocyte proliferation and that it directly induces HB-EGF, suggesting a role for PPARδ as a pathogenic factor in psoriasis. Furthermore, two regulatory aspects of PPARδ activity in keratinocytes have been defined: its independence of p65/RelA activity and localization (fig.5), and its expression independent of canonical Wnt signalling (fig.7). HB-EGF as a direct PPARδ target gene represents a highly relevant pathogenetic factor for the disease since it drives keratinocyte proliferation, regulates wound healing, and induces epidermal hyperplasia, thus replicating important elements of the psoriatic phenotype *in vivo* (Zheng *et al.*, 2003; Kimura *et al.*, 2005; Shirakata *et al.*, 2005).

The role of PPARδ on proliferation is, in fact, highly controversial and while recently it has been demonstrated that activation of PPARδ induces endothelial cell proliferation and angiogenesis (Piqueras *et al.*, 2007), other recent papers report a growth-inhibitory effect (Burdick *et al.*, 2007). In this work, however, using primary adult epidermal human keratinocytes and two different PPARδ synthetic ligands a growth-stimulatory effect was observed. Since the stimulatory time period used was seven days, the observed increase in cell number is highly unlikely to result exclusively from inhibited apoptosis. Therefore, it appears that PPARδ activation in human keratinocytes indeed enhances proliferation.

Mechanistically, PPARδ may act as an antagonist to other nuclear hormone receptor heterodimers. Thus, the RXR/RAR heterodimer activates, rather than inhibits apoptosis (Chiba et al., 1997; Monczak et al., 1997). Another competitive antagonism may be exerted toward PPARγ since activation of this isoform directly inhibits STAT3 (Wang et al., 2004) which, when overexpressed, causes a psoriasis-like phenotype *in vivo* (Sano et al., 2005). Furthermore, the anti-apoptotic activity of PPARδ may be most

pronounced in the context of type 1 interferon activity since PPARδ is expressed in dendritic cells (Jakobsen et al., 2006).

Apart from keratinocyte proliferation, the activation of type 1 IFN signaling and the persistence of activated CD8+ T cells in psoriatic epidermis are additional key features of this complex disease. In fact, PPARδ has been identified as a target gene of type 1 IFN in T cells, where PPARδ is required for protection from growth factor deprivation-induced apoptosis, but not from Fas – induced apoptosis (al Yacoub *et al.*, 2007). PPARδ may exert its pathogenic function in part by antagonizing PPARγ (Zuo *et al.*, 2006). Consistent with this hypothesis, PPARγ has a pro-apoptotic effect in T cells (Harris & Phipps, 2001, 2002), and activation of PPARγ has an inhibitory effect on psoriasis whereas this is not the case with PPARδ activation (Kuenzli & Saurat, 2003; Malhotra *et al.*, 2005).

The genes involved in fatty acid signalling as a group constitute a disease-specific determinant of PPARδ activity in psoriasis. This unexpectedly large group of genes positions intermediary metabolism as a central aspect of the disease, connected to, but distinct from its inflammatory and regenerative nature. Thus, increased body mass index is an independent risk factor for psoriasis (Naldi et al., 2005) and may have an effect on disease severity (Marino et al., 2004), as well as TNFα-related inflammatory activity (Hamminga et al., 2006). Since the prevalence of metabolic syndrome is increased in psoriasis (Sommer et al., 2006), a combination of insulin resistance, obesity, or chronic inflammation may trigger the expression of PPARδ, which in turn contributes to a non-terminated regenerative skin phenotype. This disease mechanism would be expected to be aggravated by acute inflammation, or stress via the induction of PPARδ by TNFα and stress-activated kinase (Tan et al., 2001). Clinically, these factors are precisely the most potent triggers of disease flares. Thus, activation of PPARδ in psoriasis offers a pathogenetic concept unifying various clinical aspects as well as transcriptional changes in psoriasis. Therapeutically, since caloric restriction can decrease the expression level of PPAR isoforms (Masternak et al., 2005), trials to this end appear warranted to explore the efficacy of caloric restriction as a treatment option in appropriately selected psoriasis patients. In fact, a recent report suggests that caloric restriction indeed has a favorable effect on psoriasis (Gisondi *et al.*, 2007). Conversely, currently on-going trials employing PPARδ agonists for the treatment of metabolic syndrome and obesity should be carefully scrutinized for increased incidence of psoriasis.

Additionally, it should be pointed out that the PPARδ gene is located 4.2 Mb telomeric of the putative PSORS1 locus at HLA-C on chromosome 6p21.2. Since earlier genome-wide scans were performed using microsatellites located actually closer to the PPARδ locus than to HLA-C and yielding a positive signal (Nair *et al.*, 1997; Trembath *et al.*, 1997; Enlund *et al.*, 1999), PPARδ formally constitutes a candidate susceptibility gene for the disease.

The upregulation and activity of PPARδ in lesional psoriasis suggests how the psoriasis-like phenotype in K14-VEGF transgenic mice relates to the human disease since VEGF is a target gene of PPARδ (Xia *et al.*, 2003; Sano *et al.*, 2005). It is also intriguing that, in contrast to human skin, PPARδ is not expressed in adult interfollicular epidermis, perhaps explaining why psoriasiform dermatitis does not occur spontaneously in mice. Although many various murine phenotypes have been proposed to be "psoriasis-like" none of these so far has reproduced the complex psoriatic phenotype. Availability of a suitable animal model of psoriasis would be of importance to the research into the disease pathogenesis. It could be hypothesized that overexpression of PPARδ *in vivo* would generate a phenotype resembling psoriasis with a proven link to the pathogenesis of the human disease. Accordingly, the lentiviral construct able to facilitate an inducible overexpression of functional, transcriptionaly active PPARδ would be suitable to generate a transgenic mouse line.

The 5LN-33 CAM-PPARδ/pIGETOn plasmid facilitates the expression of a VP16- PPARδ fusion protein. The VP16 fragment, in addition to ensure transcription factor activity, allows easy monitoring of the transgene expression level and its intracellular localization. Native PPARδ is imported into nucleus, but very little is known about the nuclear localizing sequence (NLS) of this protein. Based on the consensus sequence proposed by Chelsky et al. (Chelsky *et al.*, 1989): Lys-Arg/Lys-X-Arg/Lys two clusters of basic amino acids, separated by a spacer of 12 amino acids within the hinge region could be recognized as responsible for PPARδ nuclear translocation (aa 179-180 and 193-197). As the nuclear translocation of native PPARδ upon its activation observed in human primary keratinocytes (figure 4) is not very efficient, engineering of an artificial NLS sequence may be considered beneficial to investigate PPARδ function *in vivo*.

Another problem, which has to be overcome during the generating of transgenic mice line is the considerable leakiness of the tetracycline-regulated gene switches. Empirical studies are necessary to investigate whether the basal transgene expression in the absence of Dox (so called "leakiness") of the 5LN-33 CAM-PPARδ/pIGETOn

construct would influence embryo development or viability *in vivo*. If that would be the case, the tightness of the TetOn system could be substantially improved by complementing transcriptional activators with antagonizing repressors such as tTS-H4 (Bockamp *et al.*, 2007) or tTRKRAB (Szulc *et al.*, 2006).

In closing, the data presented in this thesis strongly suggest a role of PPARδ in the pathogenesis of psoriasis and lay the groundwork to address this question *in vivo* through the generation of transgenic mice.